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ON THE POSSIBILITY OF THE OCCURRENCE OF TRYPANOSOMIASIS IN INDIA.

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THE recent discovery of trypanosomiasis in man by Dr. Dutton and Dr. Forde,[1] and the report of further cases by Dr. Manson,[2] naturally lead one to question the possibility of the occurrence of this disease in other parts of the world than those originally reported—viz., the Congo and the Gambia. In the following remarks I hope to show that there is at least some ground for the belief that it may occur in India, and that a species of trypanosoma may be the cause of one of the indefinite varieties of fever occurring in that country, in which the presence of malaria parasites in the blood is not determined or is, at least, only incidentally noted.

The case upon which this theory is based belonged to such a class, whose general features I shall briefly describe before going into details with regard to the individual patient. For want of a better name I may speak of them as cases of "Dum-dum fever," because, as far as my experience goes, the patients usually came either from this cantonment or its immediate neighbourhood. This station of Dum-dum lies about seven miles from Calcutta and is notoriously unhealthy, malarial fevers of all types, dysentery, and enteric being rife. It is excessively damp, and, in the rains, is practically a morass from the fact of its lying so low; it is said to be even a few feet below the level of the Hoogley, which flows within a mile or two of the cantonments. I had a short personal acquaintance with this station in 1890, but the present remarks refer to the features of this form of Indian fever as presented by soldiers invalided on account of it from Dum-dum to the Royal Victoria Hospital, Netley, during the past three or four years. The cases were, as a rule, regarded as having been malarial in origin, and presented, on admission to Netley, an extreme degree of cachexia; it was, in

fact, the severity of this cachectic condition and the frequency of its association with Dum-dum—and, more rarely, its immediate neighbours, Calcutta and Barrackpore—which gave rise to the idea that we were dealing with a specific type of fever, whose cause was unknown. Clinically, these cases presented no very definite features distinguishing them from other and commoner forms of tropical cachexia, the chief symptoms being an irregularly remittent type of fever, grave anaemia, progressive muscular atrophy, and great enlargement of the spleen. Digestive and bowel derangements were frequent, the latter often a legacy of previous dysentery. In none of these Netley cases were malaria parasites found in the blood, nor were there any records of their having been found at an earlier stage of the disease.

The difference, then, between these cases of Dum-dum fever and other cases of tropical cachexia, associated with a low form of fever was chiefly one of degree, and this was so marked that one of the first questions one asked a patient suffering from a particularly severe form of cachexia was as to whether he had served recently at Dum-dum. Time after time the reply was either that he had been invalided directly from this station, or that he had quite recently served there. It was at the necropsy of one of these cases of Dum-dum fever that the appearances described below were noted in the spleen, which I now think to have been due to trypanosoma infection.

Private J. B., 2nd Royal Irish Rifles, aged 23, who had been invalided from Dum-dum for dysentery, was admitted to Netley in April, 1900, and died there seven months afterwards. He had not served in any other tropical country. During these seven months he presented in an extreme degree all the general features of the class of cases I have just described, and, in addition, suffered from chronic dysentery for some time after his admission, though this had improved considerably before his death. I examined his blood on many occasions, but never detected any signs of malaria. His temperature was seldom normal, but his chart showed no regular features. At the necropsy, made 38 hours after death, beyond the intense emaciation, the only gross lesions found were in the large intestine and the spleen. The walls of the colon were deeply pigmented and greatly thickened, and were covered with the cicatrices of old dysenteric ulcers; a few unhealed ulcers were found in the neighbourhood of the sigmoid flexure. The spleen was greatly enlarged, weighing 2 lb. 7 oz., and was much congested, the spleen pulp was extremely soft and friable, the organ was sterile on cultivation. 100 oz. of clear serum were removed from the peritoneal cavity.

On making smear preparations from the spleen pulp, I was struck by the curious appearance, among the spleen cells and red corpuscles, of enormous numbers of small round or oval bodies, 2 to 3 μ in diameter, which corresponded to nothing I had previously met with or had seen figured or described. They stained faintly with methylene blue and with haematein, showing with these stains a sharply contoured circular or oval shape, but no detailed structure; but, on staining them by Romanowsky's method, they were found to possess a quantity of chromatin, of a very definite and regular shape, which clearly differentiated them from blood plates or possible nuclear detritus. This chromatin appeared in the form of a more or less definitely circular mass or ring, applied to which, though apparently not in direct connexion with it, was a much smaller chromatin mass, usually in the form of a short rod set perpendicularly or at a tangent to the circumference of the larger mass. The outlines of the sphere or oval enclosing

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these masses of chromatin were only faintly visible by this method of staining. (See Fig. 1.) These little bodies were scattered freely among the cells, as a rule isolated one from the other, but here and there aggregated into clumps composed of 20 to 50 members.

As to their meaning I was at the time completely at a loss, nor could other observers, to whom I have from time to time shown them, give me any clue as to their nature. In two fatal cases of the same type of Dum-dum fever which occurred subsequently I failed to find anything of the same nature in the spleen, though in those cases this organ was not enlarged to the same extent as in the case of Private B.

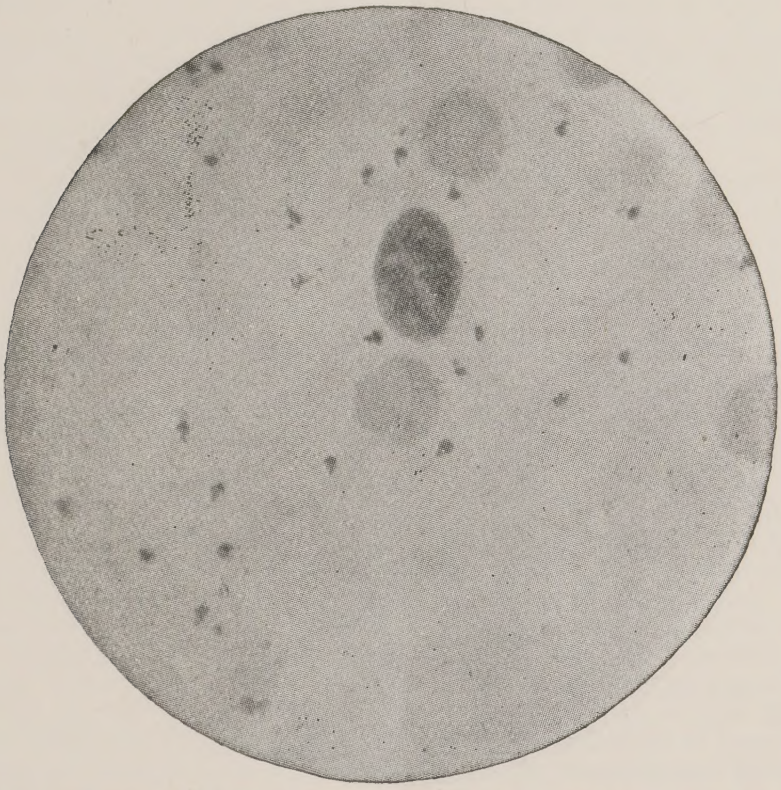

Fig. 1.—Smear preparation from the spleen of Private B., made thirty-eight hours after death and stained by Romanowsky's method. Magnification, 1,000 diameters.

It was only recently, while working with nagana, the trypanosoma of tsetse fly disease, discovered by Lieutenant-Colonel D. Bruce, R.A.M.C.,[3] that I came across the appearances in the blood and internal organs of a white rat, dead of this disease, which, I venture to think, present the key to the puzzle. On examining this animal forty-eight hours after its death, I found in the blood and organs bodies practically identical in shape and staining reaction with those I had met in the spleen of Private B. The same little circular masses of chromatin, most frequently in the form of rings of varying thickness, were seen, and, in many instances, a smaller chromatin dot or rod was noticed lying in apposition to the larger mass. As the blood of this rat on examination shortly before its death was found to be swarming with trypanosomata, there could be little doubt that these chromatin bodies

represented the macro-nuclei and micro-nuclei of the adult organisms, and that these structures were practically all that remained of the parasites, the rest of the body and the vibratile membrane, being less resistant to degenerative change, having disappeared. Further experiments soon proved this to be the case, and it was easy to trace every step in these degenerative changes from the death of the trypanosoma onwards.

The white rat, as a rule, dies about ten days after inoculation, with enormous numbers of trypanosomata in the peripheral blood, in all stages of longitudinal fission. After death the parasites very rapidly loose their motility and also their typical flagellate form, shrinking up into ovoid or

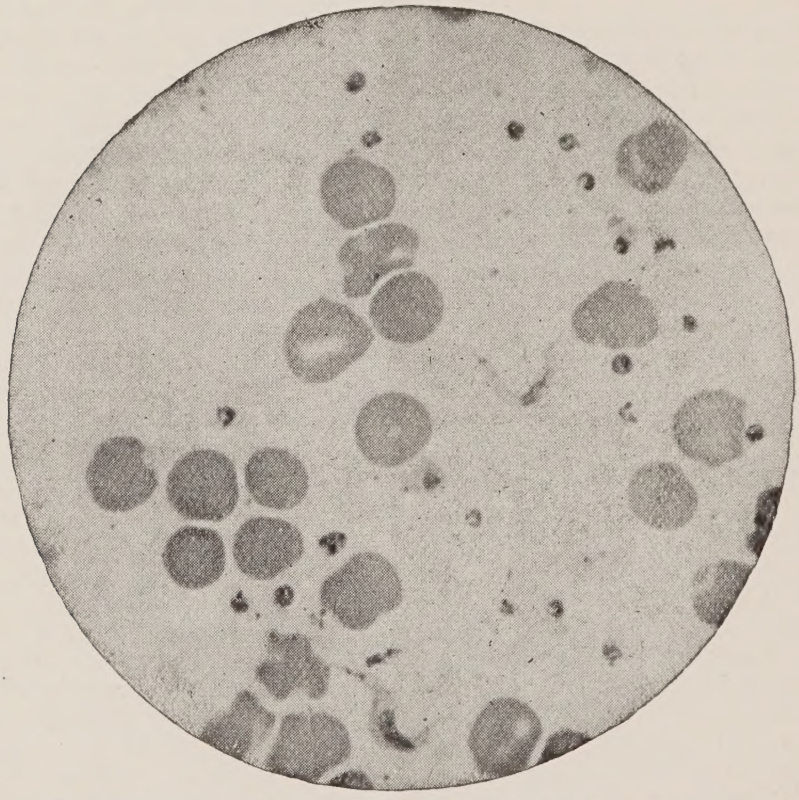

Fig. 2.—Smear preparation from the lung of a white rat, made thirty-six hours after death and stained by Romanowsky's method, showing various stages of degeneration of the trypanosomata. Magnification, 1,000 diameters.

spherical bodies, which have, to a considerable extent, lost their faculty of staining by the blue element of Romanowsky's stain. The macro-nucleus becomes denser and more circular in shape, and, together with the micro-nucleus, retains its chromatin reaction for as long as I have had the organs under observation. The vibratile membrane is rapidly shed, and, 12 hours after death, these may be seen in large numbers lying free among the dead parasites; occasionally they carry with them the micro nucleus, attached to one extremity, but, more frequently, this is left behind in the spherulated body of the parasite. Forty-eight hours after death these vibratile membranes can no longer be detected, even by prolonged staining, and the outline of the body of the parasite can only be made out in a few cases. Further shrinkage of the body approximates the macro-nucleus to the micro-nucleus, if the

latter has been left behind, and all that remains is the sharply-defined chromatin mass or ring of the altered macronucleus with, in many cases, the micro-nucleus closely applied to it, resembling in shape the young bud thrown out by a yeast cell. Fig. 2, which was taken from a lung smear 36 hours after death, shows several stages in this degenerative process.

But for the fact that the little bodies found in Private B's spleen were somewhat smaller, their staining reactions and shape corresponded almost exactly with those of the degenerated trypanosomata found in the blood and organs of the rat; and it may well be that this difference in size was due to a difference in species.

Without wishing to insist too strongly on the striking resemblance in these two conditions, I think that it at least makes out a prima facie-case in favour of the supposition that, in this particular example of Dum-dum fever, we had to deal with trypanosomiasis, if not as the actual cause of death, at least as a complication. Some other arguments may be advanced in support of this supposition.

First, we have the undoubted fact that in many of these cases of severe tropical cachexia, in particular those of Dum-dum fever, no malaria parasites can be detected either during life or, after death, in sections of the brain or internal organs, and, also, that such cases are altogether uninfluenced by quinine, arsenic or other anti-periodics. Of course such cases may incidentally contract true malaria, but, under suitable treatment, the parasites disappear, leaving the grave cachexia and increasing prostration unaffected.

Secondly, it might be urged that so obvious a parasite as a trypanosoma could scarcely be overlooked on the most cursory blood examination, and that, since such examinations are in daily practice by skilled observers at most Indian stations, the occurrence of trypanosomiasis in India would have been signalled long ere this if it really occurred in man. This certainly would be strong evidence of its non-occurrence were the parasite one which is constantly to be found in the peripheral blood, but we have only to study the progress of the disease in animals to see that a fatal result may follow inoculation without the parasites having been detected at any time in the peripheral blood. The factor which determines whether the disease shall run the course of a true septicaemia, with numerous parasites occurring and multiplying in the general circulation, or whether their presence shall be restricted to certain of the internal organs, appears to be mainly the resistance of the particular species. The experiments of Bruce,[3] Laveran and Mesnil,[4] Plimmer and Bradford,[5] and of Mantini,[6] show the widely separated limits of the time required by the trypanosoma of nagana to cause death in the different species investigated. Thus, the very susceptible rat dies in a few days, while the guinea-pig may live for several months. Speaking generally, the more resistant the species or individual, the less likely are we to find the organisms in numbers in the peripheral blood during life. Taking the white rat, again, as an example we find the parasites appearing in the blood three or four days after inoculation; they then increase enormously in numbers until, just before death, they may be almost as numerous as the red blood corpuscles. On the other hand, in the rabbit, which shows a considerably greater power of resistance, death being postponed frequently for a month or six weeks, para-

sites can only rarely be found, and frequently the most careful search of several films fails to demonstrate a single trypanosoma, the organisms in this case being found in large numbers either in some of the internal organs and the bone marrow, or forming accumulations in the lymphatics of certain regions, such as the eye or the genital organs.

It is thus evident that a fatal attack of trypanosomiasis in an animal does not necessarily postulate the occurrence of the parasites in the peripheral blood, in numbers large enough to permit of microscopical detection.

Again, the chronic nature of the disease in the few recorded cases of trypanosomiasis in man and the immunity which man appears to possess to infection by other varieties of trypanosoma, such as nagana, surra, dourine, *mal de caderas*, etc., would appear to show that man is very resistant to trypanosoma infection.

It is not, then, I think, unreasonable to assume, first, that when trypanosomiasis does occur in man the parasites are more likely to multiply and accumulate in the capillaries or lymphatics of the internal organs than to invade the general circulation in large numbers; and, secondly, that the cases which have been recently reported are in reality exceptional cases, in which the resistance of the individual was comparatively low, and in which the parasites were able to develop and multiply in the blood stream, as they do in the case of a horse, rat, or mouse infected by trypanosoma brucei.

It appears to me, therefore, as at least possible that trypanosomiasis may be a much commoner disease of tropical countries, including India, than is generally supposed, and that its non-recognition may be due, as I have suggested above, partly to the tendency of the parasite to confine itself to the internal organs during the life of its host, and partly to the rapid degeneration and loss of characteristic shape which follow on the death of the organism and render its identification, in sections or smear preparations, a matter of difficulty. This difficulty is further increased by reason of the fact that only by Romanowsky staining can the macronuclei and micro-nuclei be rendered visible, and then only in smear preparations, since it is very hard to produce satisfactory chromatin staining in sections owing to the chemical alterations induced in the tissues by the various processes of hardening and embedding. I have tried to modify these processes in various ways, bnt so far without success.

With a view, then, to finding out whether some of these severe tropical cachexias, such as the Dum-dum fever, may not be due to trypanosomiasis, I would suggest that, as a routine practice, necropsies of such cases should include the staining of smear preparations from the spleen, lungs, and liver by Romanowsky's method, and a careful search for the degeneration forms of the parasites which I have described.

Increased facilities for the detection of trypanosomiasis during life are much to be desired. Positive results might be obtained by inoculation of a susceptible animal with blood from a suspected case, although this procedure does not appear to have been successful in Dr. Manson's case, and Drs. Annett and Dutton record that the pathogenicity of the trypanosoma in their case was not marked in white rats. Possibly the monkey might prove more susceptible to the trypanosoma of man. Some of the methods of staining thick blood films by dissolving out the haemoglobin, such as those

of Laveran and of Ross, may also give an increased chance of success, and in this connexion I may note that the modification of Romanowsky's stain, described by me in the BRITISH MEDICAL JOURNAL,[7] may be utilized for this purpose by mixing a little of the stain with twice its volume of water, pouring the mixture on to the unfixed film, and at the end of three or four minutes gently washing it off, when the film may be dried and mounted. It has also been suggested that larger quantities of blood may be examined by citrating and employing the centrifuge.

I would further suggest, in conclusion, that the procedure I have indicated might possibly be of service in the investigation of kala-azar and of sleeping sickness, both of which have recently been considered as perhaps due to trypanosomiasis.

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